when the temperature was returned to 23°C. In marked contrast, conductance due to ΔF508 CFTR channels exhibited a transient increase within a minute after the temperature challenge, followed by a quasi-exponential decline of about 80-90% of the initial conductance ($t_{1/2}$ = 4 minutes at 37°C). The temperature induced decrease in ΔF508 CFTR conductance was not reversed by returning the temperature to 23°C. The second-site reverent construct, R553M/ ΔF508 CFTR, previously shown to rescue CFTR function in mammalian cells (Teem et al. 1993. *Cell*. 73:335-346), exhibited a thermal response that was indistinguishable from wild type. Preliminary data suggests that this "thermal instability" that is readily detectable when ΔF508 CFTR is expressed in *Xenopus* oocytes, reflects an intrinsic structural defect in the channel protein that results in a temperature-sensitive alteration in gating and could potentially trigger the retrieval of surface protein documented in mammalian cells.

1677-Pos
Riding the Conformational Wave to the Open Channel State in the CFTR Chloride Channel
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The pore structure of the CFTR chloride channel is unknown. We showed previously that R352 in TM6 forms a salt bridge with D993 in TM9; charge-destroying mutations at either site destabilized the open state, affecting conductance, selectivity, and pore blockade. Other pairs of interacting residues also contribute to stabilizing the open state. We continued these experiments to determine how steps leading to the dimerization of the NBDs upon binding of nucleotide relate to the steps leading to pore opening, using single-channel recordings of WT-CFTR and channels bearing a cysteine or alanine at 352, 993, or both. In R352C-CFTR, but not R352A-CFTR, modification of the cysteine by positively-charged $MTSET^+$ and $MTSEA^+$ recovered the stability of the open state. In D993C-CFTR, but not D993A-CFTR, negatively-charged $MTSES^-$ recovered the stability of the open state. In contrast, D993C-CFTR modified by $MTSET^+$ retained the instability of the open state. The R352C/D993C-CFTR double mutant exhibited instability of the open state in both the absence and presence of DTT, suggesting that R352C did not form a disulfide with D993C. In WT-CFTR, exposure to AMP-PNP led to greatly prolonged channel openings, as expected. However, this response was not found for R352A-CFTR. Surprisingly, R352C/D993C-CFTR could be latched open by the bifunctional crosslinker, MTS-2-MTS, such that channels could not close upon washout of ATP. MD simulations based on CFTR homology models (see Dawson Lab abstract) predict conformational states in which R352 and D993 approach each other to within van der Waals distances. These results suggest that the binding of ATP at CFTR's NBDs initiates a conformational wave, which leads to a change in pore structure from the closed to the open state, the latter being stabilized by inter-TM interactions including the R352-D993 salt bridge. (Support: NIH-2R56DK056481-07)

1678-Pos
Homology Modeling and Molecular Dynamics Simulation Predict Side-Chain Orientations and Conformational Changes in the Pore of the CFTR Chloride Channel
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We recently presented two homology models of the CFTR chloride channel, one based on homology to the prokaryotic ABC transporter, Sav1866, and a second based on a 5 ns molecular dynamics (MD) simulation of the first (Alexander et al, *Biochemistry* in press, 2009). Predictions for side-chain orientations were in excellent agreement with the results of a cysteine scan of transmembrane segment six (TM6) using both channel-permeant and channel-impermeant, thiol directed probes. Here we present the results of an extended MD simulation, along with the results of a cysteine scan of TM12. Scanning results confirm the model predictions for "pore-lining" and "not pore-lining" residues in TM12 and support the notion that pore narrowing prevents the reaction of deeper-lying cysteines in TM12 toward larger, thiol-directed probes like $MTSET^+$ and $MTSES^-$ when these compounds enter the channel from the outside. The extended MD simulation predicts movements of pore elements that are in agreement with previously reported results of state-dependent reactivity of a cysteine at position 337 (Norimatsu et al, *Biophysical Journal* 96(3):468a-469a, 2009), and the postulated formation of a salt-bridge between R352 (TM6) and D993 (TM9) (Cui et al, *Biophysical Journal* 91(5):1737-48, 2008, and poster from the McCarty Lab). Supported by NIH, the Cystic Fibrosis Foundation, the American Lung Association, the Wellcome Trust, and the BBSRC.

1679-Pos
Identification of Possible Binding Sites for the CFTR Pore Blocker, GlyH-101
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The last decade has seen the discovery by means of high throughput screening of a wide range of small-molecule modulators of the CFTR chloride channel. These compounds act by altering anion conduction, channel gating and/or trafficking of the CFTR protein. However, binding sites for these molecules on CFTR or other cellular constituents have yet to be identified. GlyH-101 is a CFTR modulator that blocks the channel by entering from the extracellular side and binding to a site within the pore. In an effort to identify possible GlyH-101 binding sites within the pore of the CFTR channel, we applied the small-molecule docking program, "Glide" (Schrodinger, Inc.), to a series of molecular models of CFTR, derived by means of molecular dynamics simulation from a homology model based on the prokaryotic ABC transporter, Sav1866 (Dawson and Locher, Nature 443: 180-185, 2006; Alexander et al., Biochemistry in press, 2009). One of the potential GlyH-101 binding sites identified by Glide lies in close proximity to two residues in the sixth transmembrane segment (TM6), F337 and T338, where substituted cysteines are "protected" by GlyH-101 from reaction with thiol-directed probes (Norimatsu et al., Biophys. Journal 96: 468a-469a, 2009). These results suggest an approach to identifying the binding site(s) for GlyH-101 and other small molecules within the CFTR protein. Supported by NIH, Cystic Fibrosis Foundation, American Lung Association, the Wellcome Trust, and the BBSRC.

Mechanosensitive Channels

1680-Pos
Mechanosensitivity of a Voltage-Gated Ion Channel, $Na_v1.5$
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Voltage-gated ion channels are often found in tissues where electrical and mechanical stimuli coexist. The mechanosensitive, voltage-gated sodium channel $Na_v1.5$ (encoded by SCN5A) is expressed in two such electromechanical organs, the heart and the gastrointestinal tract. Mutations in SCN5A are frequently pathogenic and may affect mechanoelectrical coupling. The aim of this study was to assess mechanical sensitivity of $Na_v1.5$ at the molecular level. SCN5A was expressed in HEK cells and studied using a pipette pulled and fire polished to ensure that a small number (2-50) of channels were reliably present in cell-attached micropatches. This allowed resolution of both single channel events and averaged behavior. Both positive and negative pressures (up to 50mmHg) produced visible patch distention, an increase in patch current at all voltages and large hyperpolarizing shifts in steady-state voltage-sensitivity of activation and inactivation. From voltage dependence of activation at rest ($V_{1/2}$ = −30mV at 0mmHg), pressure resulted in graded shifts of $V_{1/2}$ for activation and inactivation of −0.71mV/mmHg and −0.72mV/mmHg, respectively. Channel kinetics were predictably affected by the voltage shifts, but channel opening and fast inactivation were otherwise unaffected by pressure. Single channel traces showed that unitary conductance was unaffected, rather peak currents appeared to increase due to an increase in the number of active channels in the patch. These effects were minimally reversible for as long as 30 minutes after a single stretch stimulus. Patch excision resulted in an immediate shift of activation $V_{1/2}$=-75mV and loss of stretch sensitivity. Application of the inhibitor of actin polymerization, cytochalasin D, diminished sensitivity to stretch (−0.42mV/mmHg). Our work demonstrates that mechanical stress at physiologically relevant levels affects voltage sensing of $Na_v1.5$ channels, without affecting the pore, channel gate and fast inactivation. Supported by NIH DK52766.

1681-Pos
Integrin-Dependent and -Independent Potentiation of L-type Calcium Current (Cav1.2) by Cell Stretch
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Stretch-induced (myogenic) contraction of vascular smooth muscle (VSM) requires calcium influx through L-type calcium channels (Cav1.2). Integrins play a role in this process because alpha5 beta1 and alphaVbeta3 integrin blocking antibodies prevent myogenic constriction. Recent studies in our lab indicate that Cav1.2 current is potentiated by alpha5beta1 integrin activation and requires phosphorylation by PKA and c-Src of Cav1.2 C-terminal residues. To test